Characterization of bacteria with plant growth promotion and antagonistic activity associated to rhizosphere and phyllosphere of Platanus mexicana and Persea shiedeana trees natural hosts of ambrosial beetle

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Abstract

It is well established that bacteria belonging to microbiota of plants, contribute to the better development of their hosts by different mechanisms, such as, growth promotion, nutrient facilitation, stimulation of plant defenses, antagonizing pathogens or pest, and some of them are also, some microorganisms show enzymatic activities with biotechnological application in the agricultural and industrial sector. In the present study we identified and characterized fourteen bacterial strains isolated from the rhizosphere and phyllosphere of Platanus mexicana and Persea schiedeana trees; the aim of this research was to evaluate bacterial biological activities over plant growth promotion on Arabidopsis thaliana seedlings and antagonistic activity over phytopathogenic fungus Fusarium sp., besides studying their lytic ability when confronted with cellulose, pectin, or chitin as carbon sources. Such strains were classified into Curtobacterium, Plantibacter, Bacillus, Brevibacterium, Carnobacterium, Staphylococcus, Erwinia, Serratia, Exiguobacterium and Yersinia genera. Every single strain revealed at least one of the evaluated characteristics. Yersinia sp. strain PsH3-014(14D) and Bacillus sp. strain Hay2-01H(7) stand out from the other strains due to their capacity to promote plant growth in A. thaliana seedlings as well as antagonist activity against of Fusarium sp.; moreover, PsH3-014(14D) also degrades pectin and chitin, while Hay2-01H(7) degrades cellulose and pectin. In contrast, Carnobacterium gallinarum strain Chi2-3Ri was detrimental for the development of Arabidopsis seedlings but it can degrade cellulose. Erwinia sp. strain Hay2-1H was the only strain capable to degrade all three biopolymers tested (cellulose, pectin, and chitin). Further research could be directed towards the isolation and characterization of key enzymes produced by the referred strains, as well as further exploration of other metabolic capacities.

Introduction

Plants live in association with complex and diverse microbial communities whose cooperative and competitive interactions shape their own assemblages, abundance, and permanence on the plant tissues [1]. Among those microorganisms, bacteria are numerically the most abundant and diverse, not only from a phylogenetic perspective, but also from a metabolic point of view [2]. Bacteria can promote plant healthy and growth through several mechanisms: for example, by supplying nutrients, by nitrogen fixation [3] or nitrogen oxidation, solubilization of phosphate [4], siderophore production for iron sequestration. Also, bacteria can produce “plant hormones”, signaling molecules that elicit systemic resistance in plants [5]. Moreover, some bacteria outcompete, antagonize, or inhibit plant pathogens [6, 7], and can produce enzymes which transform complex and inaccessible substrates into available ones [8, 9].

Bacteria inhabit the rhizosphere and other plant organs such as leaves [10], stems or fruits [11]. Bacteria inhabiting the phyllosphere (leaf surface and inward into the leaf tissues) frequently belong to the Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria phyla [12]. Some of the bacterial community members are methylotrophic, nitrogen fixers while others strongly influence the resistance of the plant host against phytopathogens, either by microbe-microbe interactions or by stimulating the plant defense mechanisms [10]. Bacteria inhabiting the rhizosphere, called rhizobacteria, boost plant healthy development by different means, for example: by improving soil texture, pH and humidity, nutrient

availability, and secreting extracellular molecules such as hormones or secondary metabolites, antibiotics, and various signaling compounds which besides modifying the plants response, they also have an impact in the neighboring microorganisms [13]. They also produce exoenzymes which start the degradation of biopolymers facilitating the release of monomers useful for both plants and adjacent microbiota [14].

Plant growth promoting rhizobacteria (PGPR) are particularly able to promote plant growth [15] which makes them of special interest for agriculture. Some PGPR produce phytohormones like auxin, cytokinin, and gibberellin which modulate endogenous hormone levels in the plant [11]; others, enhance the plant host resistance against pathogen infection either through commensal-pathogen interactions [16, 17] or through modulating plant defense [18, 5]. Production of different anthocyanins has been recognized as a plant response to different abiotic stresses as drought, high salinity, low temperature, and excess light [19] or in responses to biotic stress, able to trigger induced systemic resistance [20, 21] against to fungi and bacteria phytopathogenic.

Biocontrol agents formulated with plant growth promoting bacteria are equipped to perform other functions besides being phytopathogen or antagonists. They also bring about plant growth promotion, soil biofertilization, abiotic stress or drought mitigation [22]. *Fusarium solani* is among the phytopathogens causing disease in many agriculturally important crops; its biocontrol has been accomplished applying bacteria from the genus *Bacillus, Acinetobacter, Streptomyces, Pseudomonas* as antagonists [23, 24, 6, 22]. Some of the bacterial mechanisms involved in the antagonism as antibiosis, competition, induced systemic resistance of the host plant, siderophore production, and even hydrolytic enzyme production [14]. The microorganism able to produce chitinolytic enzymes such as chitinases, play an important role as biocontrol agents and pathogen antagonists [7].

In the present study, we isolated, identified and characterized bacteria from the rhizosphere and phyllosphere of *Platanus mexicana* and *Persea schiedeana* trees, natural hosts of ambrosial beetles, and evaluated its biological activities over *Arabidopsis thaliana* seedlings and antagonic activity against phytopathogenic fungus *Fusarium sp.*, INECOL_BM-06, a phytopathogenic fungus associated to *Xylosandrus morigerus* ambrosia beetle[34], as well as enzymatic activity properties. Our results show that bacteria, PsH3-014(14D) isolated from *P. shiedeana* and Hay2-01H(7) from *P. mexicana*, promote plant growth in Arabidopsis seedlings and antagonistic activity against the phytopathogenic fungus *Fusarium sp*. Our results suggest the biological potential of host-associated microorganisms with potential biological activities in the biological control of diseases and pest, as a natural defense mechanism of the host plant against attack by a phytopathogen agent. Considering the broad spectrum of bacterial activities, found in the plant microbiome, looking for strains with potential biotechnological application in agriculture or industry related to the transformation of agro-industrial residues, or the transformation of complex carbohydrates.

Materials And Methods

2.1 Isolation of bacterial strains

Root, bark and leave samples from *Platanus mexicana* and *Persea schiedeana* trees, were collected in the “Foggy Forest Sanctuary” (19°30'47.5"N; 96°56'11.5"W) of the Protected Natural Area “Francisco Javier Clavijero” of the Institute of Ecology (INECOL) in the city of Xalapa, México. Such samples were handled and processed with sterile materials and then processed in the laboratory under axenic conditions. Five grams of each sample type; root, bark or leave, were independently processed. Each tissue sample, was first rinsed with alcohol (96%) for one minute, followed by two subsequent one-minute rinses with sterile water; then, they were grinded and resuspended in 5 mL of a sodium phosphate solution (0.9%). Two subsequent serial dilutions (1:10) were prepared and separated bacterial colonies, were obtained from the last dilution by using the cross-streak method on Luria-Bertani (LB) solid media, bark solid media (prepared with LB media (50%) plus bark extract (50%)) and V8 agar plates. Bacteria with distinctive colony morphology, were selected for purification and characterization. Purity was confirmed by microscopic observations of the colony morphology using a Leica S8 APO stereoscopic microscope, and determining the cell morphology with Gram stain reaction, using a Leica DM750 light microscope.

2.2 *Plant material and growth conditions*

Arabidopsis thaliana seeds ecotype Columbia-0 (Col-0) were used for the present study. *Arabidopsis* seeds were superficially disinfected with 700 µL of 96% (v/v) ethanol for 7 min, followed by a bleach wash at 20% (v/v) for 7 min. Finally, six washes were performed with sterile distilled water and stored at 4°C for 48 hours. *Arabidopsis* seeds were germinated and grown in 0.2x Murashige and Skoog (MS) agar medium and incubated in a plant growth chamber (Percival AR36L model) with a photoperiod of 16 h light and 8 h dark, with a luminous intensity of 100 µmol photons m^{-2} s^{-1} and a temperature of 22 ºC.

2.3 DNA extraction, 16S rRNA gen amplification and phylogenetic identification

Biomass of each isolated and pure bacterial strain was collected on a 1.5 mL Eppendorf centrifuge tube, either from 1 mL of liquid culture or from a single colony from solid culture; then it was centrifuged at 3000 rpm for 5 minutes and rinsed with an isotonic NaCl solution (0.9%) twice, removing the liquid and keeping the rinsed cells pellet. DNA extraction was performed with the following procedure: 160 µL of solution I (Tris base 10 mM, NaCl 60 mM, Sucrose 5%, EDTA 10 mM) were added to the pellet, mixed well and the suspended cells were lysed by freezing (-80°C) then heating them (~ 90°C). Afterwards, 200 µL of solution II (Tris base 300 mM, Sodium Dodecyl Sulfate 1.25%, Sucrose 5%, EDTA 10 mM) were added and the mix was incubated 10 min at 65°C; subsequently 60 µL of solution III (Potassium Acetate 3M), vortexed and centrifuged (15,000 rpm by 10 min) finally, 200 µL of supernatant were transferred to a clean 1.5 mL Eppendorf tube. The supernatant was then mixed with 420 µL of isopropanol and incubated at room temperature for 5 min; followed by centrifugation at 15,000 rpm for 10 min; supernatant is removed to keep the resulting DNA pellet, which is then rinsed twice, with cold alcohol (70%) for resuspension with sterile double distilled water, for quantification and integrity and purity analyses for

further processing. The 16SrRNA gene of each strain was amplified by 30 cycles at standard PCR conditions and reaction mix, using 27F (3´-AGAGTTTGATCCTGGCTCAG-5´) and 1492R (3´-TACCTTGTTACGACTT-5´) primers and 48°C for 30 s as annealing temperature. The PCR products were sequenced by the LABSERGEN capillary sequencing service unit, CINVESTAV, Irapuato, México.

For the phylogenetic identification, sequences were first revised with DECIPHER (v2.0) to discard chimeric sequences [25] and were further analyzed using the Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) [26] and the free trial of Geneious, Bioinformatics Software for Sequence Data Analysis [27]. To construct the phylogenetic trees, the closet homologous sequences used, were downloaded from the National Center for Biotechnology Information with the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) restricted to type strain material; plus, sequences found at the SILVA rRNA database project [28]. The classification was further confirmed by using the Ribosomal Database Project (RDP) tools [29]. The evolutionary history was inferred by the Maximum Likelihood Method and Tamura-Nei Model [30]. 16S rDNA sequences obtained were deposited in GenBank (Accession numbers: OL449779-OL449799).

2.4 Arabidopsis-bacteria *in vitro* co-inoculation system

Bacterial isolated from *P. mexicana* and *P. schiedeana* were evaluated *in vitro* for their plant growth-promotion ability, using *Arabidopsis* seedlings (Col-0) ecotype [31]. Five-day-old after germination ten seedlings of *Arabidopsis* wild type (Col-0) were transferred to 0.2x MS agar medium and were co-inoculated by streaking on agar medium and grown for further 8 days period by placing the Petri dishes in the plant growth chamber (Percival AR36L) under the conditions mentioned above. *Pseudomonas aeruginosa* PAO1 wild type and *P. aeruginosa LasI*-, a quorum-sensing (QS) mutant affected in the production QS signal, were used as negative and positive, respectively [32]. All experiments were replicated at least three times.

2.5 Biomass and anthocyanin content

After the 8 days of co-cultivated, representative images were photographed and shoot fresh weight was recorded by using an analytic balance (Ohaus, Explorer) with a 0.0001 precision valued. For the anthocyanin content was determined according to Tzec-Interian *et al*, [33]. All experiments were replicate at least twice times.

2.6 Antagonistic activity of bacterial strains against phytopathogenic fungus Fusarium sp.

To determine if the bacterial strains had an antagonistic effect against the growth of *Fusarium sp.* pathogenic strain INECOL_BM-06 provided by the Phytopathology Laboratory at Institute of Ecology, A.C. (INECOL) [34]; both organisms were co-cultured in Petri plates of Potato Dextrose Agar (PDA) media, three petri plates, per bacterial strain plus three petri plates only with *Fusarium sp.* as absolute growth control, which we consider “negative control”. 10 µL of a conidia suspension ($1x10^5$ conidia/mL) were placed at the center of the petri dish, and 3 drops with 10 µL of a bacterial liquid culture (OD_{600} = 0.1) were placed

in 3 out of 4 quadrants (at 2 mm from the border of the petri plate); the 4^{rth} quadrant received 10 µL of LB liquid media as to serve as diffusible compounds negative reference. The petri dishes were incubated for 9 days at 28°C. Growth inhibition (I) in percentage was calculated applying the following formula: **I=[(R-r)/R] x 100** where **R** is the radius of *Fusarium sp.* growth in the negative control petri plates and **r** is the radius of growth of *Fusarium sp.* in the direction of the bacterial strain evaluated. ANOVA ($\alpha \leq 0.05$) statistical analyses were performed with media averages and standard deviations of the data.

2.7 Enzymatic activities of bacterial strains

To qualitatively evaluate, each bacteria capacity to degrade either cellulose, pectin, or chitin; 10 µL of each bacterial liquid culture (OD_{600} = 0.1) were placed in the center of the well containing differential culturing media and were incubated at 28°C for 48 h, to posteriorly evaluate (with the corresponding revelation reagent) if the tested biopolymer was transformed during the growth of the strain. Corning® Costar® 24 microwell plates containing 1.6 mL of a differential media (either with cellulose, pectin, or chitin). The experimental design included three replicates (One well corresponding to one replicate) of: each strain, the negative control (sterile media, not inoculated) and the positive control (sterile media, inoculated with an active strain, able to degrade the evaluated polymer). Each differential media was prepared, and the degradation was revelated as follows:

2.7.1 Cellulolytic activity

Cellulolytic activity was evaluated with Cellulose Agar, each liter of which, was prepared by adding 5 mL of stock saline solution (K_2HPO_4 [5 g/L], $MgSO_4$ [2.5 g/L], NaCl [2.5 g/L], $Fe_2(SO_4)_3$ [0.5 g/L]), 1 mL of micronutrient stock solution (K_2MoO_4 [0.05 g/L], $MnSO_4$ [0.05 g/L], Na_3BO_3 [0.05 g/L], $CoNO_3$ [0.05 g/L], Cu_2SO_4 [0.05 g/L]), 1 g of NH_4NO_3 and the pH was adjusted to 6.5 prior to add carboxymethyl cellulose (10 g mixed with blender with 100 mL of distilled water), plus 15 g of agar. Finally, it was submitted to moist heat sterilization, and poured into the Corning® Costar® 24 microwell plates. After the proper incubation period for the growth of the bacteria, the degradation of cellulose was revealed by flooding each microwell, with 300 µL of Congo Red [1%], incubating at room temperature for 15 minutes, then removing the excess of Congo Red and afterwards, rinsing with 300 µL of NaCl [1M] allowing 15 more minutes to pass before registering the result. The presence of a clear zone surrounding the bacterial growth area, indicated degradation of cellulose; when the media was dyed in red without a clear halo, it indicated a negative result.

2.7.2 Pectinolytic activity

The degradation of pectin involves the activity of different enzymes which work at different pH, we evaluated it at pH 5 and pH 7, using pectin agar. Each liter of “Pectin Agar”, had 30 g of agar, plus a 1:1 mixture of solution A, and solution B adjusted to pH 7 or pH 5 accordingly. Both A and B solutions, were premade and stored at 5°C. Each liter of solution A contained 2 g of yeast extract and 10 g of apple (or orange) pectin. One liter of solution B had 2 g of $(NH_4)_2SO_4$, 4 g of KH_2PO_4, 6 g of Na_2HPO_4 and 1 mL of Stock Solution BC ($FeSO_4 \cdot 7H_2O$ [0.1 g/L], $CaCl_2$ [1 g/L], H_3BO_3 [0.01 g/L], $MnSO_4$ [0.07 g/L], $CuSO_4$ [0.05

g/L], MoO_3 [0.01 g/L]). To develop the presence or absence of pectin degradation, 300 µL of Lugol's iodine, were added to each well to completely cover the media surface; after 5 minutes, the excess of the iodine was removed, and the absence of brown pigmentation surrounding the area of bacterial growth, was indicative of pectin degradation, a complete brown surface indicated no degradation.

2.7.3 Chitinolytic activity

Chitin degradation was evaluated by detecting a pH color change in a liquid, modified basal media supplemented with pretreated chitin and bromocresol purple ($C_{21}H_{16}Br_2O_5S$) as pH indicator (which is yellow at 5.2 and purple at above 6.8). Per liter of "chitin media" containing 0.3 g of $MgSO_4 \cdot 7H_2O$, 3.0g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 1 g of monohydrated citric acid, 4.5 g of pretreated colloidal chitin, 0.15 g of bromocresol purple; then, with sodium acetate buffer, the pH was adjusted to 2.8 to finally submit the media to moist heat sterilization. The reaction was considered as positive, when the media turned from yellow to purple, after the proper incubation to allow bacterial growth, when the bacteria grew but the media didn't change its color, the reaction was considered as negative. The pre-treatment of chitin consisted of socking 10 g of commercial colloidal chitin in 100 mL of phosphoric acid [85%], and left during 24h at 4 ^{o}C (with sporadic shaking); after such time, it was washed with abundant distilled water and filtered, until it reached pH 7, afterwards it was sterilized by moist heat, and stored at 4 C

2.8 Data analysis

For all experiments, the overall data were statistically analyzed in the SPSS 10 program (SPSS, Chicago, IL, USA). Univariable analyses with a Tukey´s post hoc test was used for testing differences in shoot biomass and anthocyanin content. Different letters were used to indicate means that differ significantly ($P < 0.05$).

Results

3.1 Bacterial strains characteristics and molecular identification

A total of 20 bacterial strains were obtained from the root, bark and leaves of *Persea schiedeana* and 32 bacterial strains from *Platanus mexicana*. We evaluate the *in vitro* effects of these 52 strains by using Arabidopsis-bacteria system to identify bacteria with potential plant growth-promotion activity in *Arabidopsis* seedlings. We identified six strains from *Persea schiedeana* Chi 0-1C (32D), PsC1-004 (35i), PsH3-014(14D), Chi 3–5(23), Chi 2-3Ri, and Chi 4-1Ri(68i) with plant growth promotion activity, and eight strains from *Platanus mexicana* the strains PmC1-001, Hay 2-11C (35i), Hay 2-01H (7), Hay 2-1H, PmH2-008, Hay 2-5R, PmRi2-006 and PmRi3-012 shows plant growth promotion in Arabidopsis seedlings. Analysis of partial sequences of their 16S rRNA gene indicated that nine of the fourteen selected bacteria, belong to the Firmicutes Phylum, three are Proteobacteria, and only two are Actinobacteria. Their closest homologous sequences were included in the construction of the phylogenetic tree which illustrates their molecular identification (Fig. 1).

According to the phylogenetic analysis, five different genera were identified among the *Persea schiedeana* isolates corresponding to *Brevibacterium frigoritolerans*, *Curtobacterium pusillum*, *Yersinia* sp., *Serratia grimesii* and *Carnobacterium gallinarum.* The *Platanus mexicana* isolates corresponded to *Staphylococcus saprophyticus*, *Plantibacter* sp., *Bacillus* sp., *Erwinia* sp. and *Exiguobacterium* sp. (Table 1).

3.2 Plant growth-promoting activity of bacteria associated to P. shiedeana and P. mexicana trees.

We evaluated the *in vitro* effects of bacteria associated to *P. shiedeana* and *P. mexicana* by using an Arabidopsis-bacteria co-culture system to elucidated the potential plant growth-promotion activity in Arabidopsis seedlings. *Pseudomonas aeruginosa* PAO1 (Wild Type) and *LasI-* (a mutant affected in the production of the quorum-sensing signal 3-oxo-dodecanoyl-homoserine lactone) were used as control negative and positive in the plant growth promotion (Figs. 2 and 3).

All bacteria strains, except for the Chi2-3Ri, had a plant growth-promoting effect in the Arabidopsis seedlings co-culture, these effects are evidence by an increase in the lateral root number and biomass as shoot fresh weight (Fig. 2B and C) in the same way to the effects shown by Arabidopsis seedlings co-culture with *P. aeruginosa LasI-* compared with uninoculated Arabidopsis seedlings (Figs. 2 & 3).

The co-culture effects of bacteria on primary root growth shows differential responses. Bacterial strains associated with *P. schiedeana* (Chi0-1C(32D), PsC1-004(35i), PsH3-014(14D) and Chi4-1Ri(68i)) and PmRi3-012 of *P. mexicana*, shows a beneficial effect on primary root growth similar to the effects in Arabidopsis seedlings co-inoculated with *P. aeruginosa LasI-*. Besides, the Chi2-3Ri isolated from *P. schiedeana* and PmC1-001, Hay2-11C(35i), Hay2-01H(7), Hay2-1(35i), PmH2-008, Hay2-5R, PmRi2-006 isolated from *P. mexicana*, shows an inhibitory effects in the primary root length similar to effects showed in Arabidopsis seedlings co-inoculated with P. aeruginosa PAO1 (Fig. 2A & 3).

Plant stress caused by co-inoculation with bacterial strains was minimal, only *Arabidopsis* seedlings co-inoculated with Chi2-3Ri induces the accumulation of anthocyanins four times more than those inoculated with *Pseudomonas aeruginosa* PAO1, a bacterial strain used as phytopathogenic strain, a minimal quantity of anthocyanins was stimulated by the growth of Chi 3–5(23), Hay 2-1H and PmH2-008 in contact with the seedlings (Fig. 2D).

3.3 Antagonistic effects of bacterial strains against phytopathogenic fungus Fusarium sp. strain INECOL_BM-06.

To evaluated the antagonistic activity of bacteria strains isolated from *P. schiedeana* and *P. mexicana* against phytopathogenic fungus *Fusarium* sp., we evaluated the inhibitory radial growth of Fusarium sp. strain INECOL_BM-06 a phytopathogenic fungus [34]. We found that the strains PsH3-014(14D) and Hay2-01H(7) caused a significant growth inhibition to *Fusarium sp.* strain INECOL_BM-06 corresponding to 37.2% and 45.5% respectively (Fig. 4D, J), compared with control growth of *Fusarium* sp. strain (Fig. 4A). However, the mechanism for such inhibition, seems to be different, considering the fungal

growth shape during the *in vitro* co-culture; a speculative suggestion is that diffusible elements were produced by Hay2-01H(7), while volatiles could have been produced by PsH3-014(14D) but further test need to be elaborated, to test those possibilities or find new ones.

3.4 Enzymatic activities of bacterial strains

Bacteria represent an important source of enzymatic biological activities with diverse biotechnological applications. In order to identify enzymatic activities of the strains isolated from *Platanus mexicana* and *Persea schiedeana* trees. We evaluated the enzymatic activity of degraded cellulose, chitine and pectine. From strains isolated from *P. mexicana* and *P. schiedeana* only nine bacteria (*Brevibacterium frigoritolerans* Chi 0-1C(32D), *Serratia grimesii* Chi 3–5(23), *Carnobacterium gallinarum* Chi 2-3Ri, *Plantibacter* sp. Hay 2-11C(35i), *Bacillus* sp. Hay 2-01H(7), *Erwinia* sp. Hay 2-1H, *Bacillus* sp. PmH2-008, *Bacillus* sp. Hay 2-5R, *Exiguobacterium* sp. PmRi3-012) were able to degrade cellulose; in contrast, seven bacteria (Yersinia sp. PsH3-014(14D); *Staphylococcus saprophyticus* PmC1-001; *Plantibacter* sp. Hay2-11C(35i); *Bacillus* sp. Hay2-01H(7); *Erwinia* sp. Hay2-1H; *Bacillus* sp. Hay2-5R; *Exiguobacterium* sp. PmRi3-012) revealed to have pectinolytic activities at pH 5 and pH7; and only one *Carnobacterium gallinarum* Chi4-1Ri(68i) at pH7 (Table 1). Additionally, only two strains *Yersenia* sp. PsH3-014(14D) and *Erwinia* sp. Hay 2-1H were able to degrade chitin. *Yersinia* sp. PsH3-014(14D) was able to degrade chitin and pectin (at pH 5 and pH 7). Only strain *Erwinia* sp. Hay 2-1H has cellulolytic, pectinolytic and chitinolytic activities. Since the assays were qualitative, further experiments would be adequate to quantify the substrates degradation efficacy of each strain, as well as further protein research would be relevant to determine the nature of the enzymes being produced by each strain.

Discussion

Given the biotechnological potential that bacteria isolated from various habitats in the world have shown, it is imperative to study the microbiota that tropical montane cloud forests in Mexico, since they are extremely diverse ecosystems but threatened by deforestation and land use change [35]. For this reason, several research groups have focused on isolating bacteria associated to forest species [36] aiming for different applications, such as biocontrol of phytopathogens [37, 38]. In the present study, the biotechnological potential of fourteen bacteria isolated from the rhizosphere and phyllosphere of two species of trees from the tropical montane cloud forests (*Persea schiedeana* and *Platanus mexicana*) were evaluated, some of them presented enzymatic activities of cellulases, pectinases and chitinases, as well as biocontrol capacity against *Fusarium sp.* as well as promote the growth of *A. thaliana* seedlings.

The biological activity revealed by each of the isolated bacteria was congruent to previously detected characteristics according to the genus to which they belong; the strains Hay2-11C(35i), PmRi3-012, and PmC1-001, PmRi2-006 identified as *Plantibacter* sp., *Exiguobacterium* sp. and *Staphylococcus saprophyticus* respectively, demonstrated the ability to promote the growth of *Arabidopsis* seedlings, but were not capable of inhibiting the growth of *Fusarium* sp. These characteristics are coherent with what is described in the literature, for example, Mayer *et al.*,[39] determined that *Plantibacter flavus* strain M259 promoted *Arabidopsis* shoot and root growth, as well as root growth in lettuce and stimulated the shoot

growth of bok choy seedlings. The *Exiguobacterium* genus has an important adaptability to various extreme environments, some strains have been studied for their ability to promote plant growth through several direct and indirect mechanisms [40]. Santos and Rigobelo [41] detailed that the strain IJ8 of *Staphylococcus saprophyticus* isolated from sugar cane, was able to produce 45.3 μg mL^{-1} of the phytohormone IAA, it was able to fix nitrogen and solubilize phosphate as well as presented cellulolytic activity.

Likewise, bacterial strains belonging to the genera *Carnobacterium gallinarum* Chi2-3Ri and Chi 4-1Ri(68i), *Curtobacterium pusillum* PsC1-004(35i), and *Brevibacterium frigoritolerans* (Chi 0-1C(32D) were identified. Bacteria from those genera have been reported for their ability to promote the growth of corn, pepper, and other crops of agricultural importance, since they are capable of producing indole acetic acid (IAA) and solubilizing phosphorus [42, 43, 33]. In addition, they can inhibit the growth of phytopathogens such as *Fusarium verticillioides*, *Fusarium* sp., and *Colletotrichum* sp., through the production of compounds such as phenazines [44, 15].

Three strains isolated from leaves and root of *Platanus mexicana* (Hay 2-01H(7), PmH2-008 and Hay 2-5R), were classified as *Bacillus* sp., and showed cellulolytic and pectinolytic activities. *Bacillus* is one of the most widely used genera in the field and at the industrial level due to its ability to produce lytic enzymes, that can be exploited for the biodegradation of complex substrates rich in cellulose and pectin [45, 46]. Also, due to this quality, it is widely used as a biocontrol agent against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Fusarium oxysporum* [6]. Regarding the impact of *Bacillus* sp. Hay 2-01H(7), and *Bacillus* sp. Hay 2-5R over *A. thaliana* both had a growth promoting effect, mainly stimulating lateral root formation, causing an increment in the shoot biomass, of 67.7%, and 30.5% respectively. *Bacillus* sp. PmH2-008 had little promoting effect with only a 7% increment in shoot biomass yet, it stimulated the plants defense system causing a slight increase in anthocyanins production at the leaves (Fig. 3).

It is also important to highlight the enzyme capacity found for the strains identified as *Carnobacterium gallinarum* Chi2-3Ri and Chi 4-1Ri(68i) and *Exiguobacterium* sp. PmRi3-012. Strain Chi2-3Ri was positive to cellulolytic activity and strain Chi 4-1Ri(68i) to pectinolytic activity only at pH 7. Genomic analysis of *Carnobacterium* strains revealed the presence of glycoside hydrolase domains [47] which could correspond to different enzymatic activities such as those evaluated in the present study. PmRi3-012 strain degrades cellulose and pectin (at pH 5 and pH 7) which is congruent with results of others studies reporting other *Exiguobacterium* strains, with demonstrated capacity to transform complex organic and inorganic polymers [48, 40].

In this study it was possible to identify bacterial genera (*Yersinia* and *Erwinia*) that are reported in the literature as entomopathogenic and could even cause diseases in plants [49, 50]; however, according to the tests carried out, we were able to evaluate its enzyme-producing potential, antagonistic capacity, and growth stimulation in seeds. *Yersinia* sp. PsH3-014(14D) isolated from the leaves of *Persea schiedeana* was able to degrade chitin and pectin from a Petri dish, promoted the growth of *A. thaliana* seedlings

evident as an increment in the shoot biomass as well as an increased number of lateral roots compared to the sterile controls, and it also exhibited an in vitro antagonistic effect against the *Fusarium* sp. strain INECOL_BM-06 by inhibiting 37.21% the radial growth of the fungus. We did not find other reports referring to antifungal or growth promoting activities in *Yersinia* species, which is an interesting finding of our study. In the literature, various species of *Yersinia* (*Y. entomophaga* and *Y. enterocolitica*) are reported as producers of insecticidal toxin complex and enzymes (chitinases, pectate lyases, polygalacturonate lyase), that can be used for the biocontrol of coleopteran, lepidopteran, and orthopteran species [51, 52, 53, 54].

Strain Hay2-1H was identified as *Erwinia* sp. and shows ability to degrade cellulose and pectin substrates. In a previous work show that *Erwinia* species produces cellulases, chitinases and pectinases, for example *E. chrysanthemi* and *E. carotovora* [53]. Interestingly, our results with Hay2-1H, showed growth promotion in *A. thaliana* reflected as an increment in shoot biomass which was accompanied with an increment in anthocyanins content in leaves and lateral root formation in comparison to the sterile control in the in vitro assays; as well as a slight antagonistic effect against *Fusarium* sp. strain INECOL_BM-06 causing 10% inhibition in the fungal growth.

Finally, strain Chi 3–5(23) classified as *Serratia grimesii*, a Proteobacteria able to promote growth of *A. thaliana* seedlings noticeable as an increment in the lateral root formation, as well as in the green shoot biomass 18.4%; the same strain, had little impact in the growth of *Fusarium* sp., strain INECOL_BM-06, causing a reduction of 11.3% in its growth. This proteobacteria also broke cellulose. Further characterization of Chi 3–5(23) might reveal more of its traits; as those of *Serratia grimesii* strain BXF1 (isolated from the pinewood nematode that causes pine wilt disease), a strain with possesses fungal and bacterial antagonistic activities; as well as plant growth regulation and the modulation of nematode development [55].

All the strains characterized in the present study have application potential either in agriculture, or in industry; given that, they can be incorporated as bioinoculants to stimulate the growth of plants, or to reduce the impact of phytopathogenic fungus *Fusarium sp.*, furthermore, they can be cultured in bioreactors to produce enzymes efficient in the transformation of different materials with cellulose, pectin, or chitin composition such as the textile industry, processing of cellulosic or chitin rich residues, an others. Additional research could be directed towards the characterization of such enzymes, as well as further exploring other metabolic capacities of the referred strains.

Conclusion

Fourteen bacterial strains isolated from the rhizosphere, the philosopher, and the bark of *Platanus mexicana* and *Persea schiedeana*, identified as *Curtobacterium, Plantibacter, Bacillus, Brevibacterium, Carnobacterium, Staphylococcus, Erwinia, Serratia, Exiguobacterium* and *Yersinia* genera. All of them have potential application either in agriculture, or in industry, For its enzymatic capacity, biocontrol, and plant growth promotion. Highlighting the importance of isolating bacteria from the mountain cloud forest

with biotechnological applications. Several strains possessed miscellaneous capacities; strains of *Yersinia* sp. and *Bacillus* sp. stand out by their capacity to promote growth in *A. thaliana* seedlings as well as hinder the growth of *Fusarium*; and degrade at least one of the tested biopolymers. In the other hand, *Erwinia* sp. was the only strain capable to degrade cellulose, pectin, and chitin. Future research could include the characterization of the produced enzymes, as well as further exploration of more metabolic capacities of the referred strains.

Declarations

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Author contributions

R.O.-C., A.A. and R.F.-C. sourced the funding, provided the resources, and conceived the project, B.A.A.-G. and G.E.C.-R. performed the experiments and analyzed the data, O.F.-R. and R.O.-C. supervised the research and drafted the original manuscript and figures. D.H.-M. instructed B.A.A.-G. how to perform analysis to evaluate degradation activities. D.H.-M., A.A., R.F.-C. and R.O.-C. reviewed and edited the paper.

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Compliance with ethical requirements

This article does not contain any studies with human or animal subjects.

Declaration of competing interest

The authors have declared no conflict of interest.

References

1. Hassani, M.A., Durán, P., Hacquard, S., 2018. Microbial interactions within the plant holobiont. Microbiome 6, 58.

2. Kopecky, J., Samkova, Z., Sarikhani, E., Kyselková, M., Omelka, M., Kristufek, V., Divis, J., Grundmann, G.G., Moënne-Loccoz, Y., Sagova-Mareckova, M., 2019. Bacterial, archaeal and micro-eukaryotic communities characterize a disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab. Sci. Rep. 9, 14883.
3. Carvalho, T.L.G., Balsemão-Pires, E., Saraiva, R.M., Ferreira, P.C.G., Hemerly, A.S., 2014. Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. J. Exp. Bot. 65,5631–42.
4. Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2, 587.
5. Viswanath, G., Sekar, J., Prabavathy, V.R., 2016. Acyl homoserine lactone-producing rhizobacteria elicit systemic resistance in plants. Microbial-mediated induced systemic resistance in plants. Devendra K. Choudhary, Ajit Varma (Editors) Springer. pp. 135–146.
6. Khalil, M.M.R., Fierro-Coronado, R.A., Peñuelas-Rubio, O., Villa-Lerma, A.G., Plascencia-Jatomea, R., Félix-Gastélum, R., 2021. Rhizospheric bacteria as potential biocontrol agents against Fusarium wilt and crown and root rot diseases in tomato. Saudi J. Biol. Sci. 28, 7460–71.
7. Veliz, E.A., Martínez-Hidalgo, P., Hirsch, A.M., 2017. Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiol. 3, 689–705.
8. Agostoni, M., Hangasky, J.A., Marletta, M.A., 2017. Physiological and molecular understanding of bacterial polysaccharide monooxygenases. Microbiol. Mol. Biol. Rev. 81(3), e00015-17.
9. Merlin, C., Besaury, L., Niepceron, M., Mchergui, C., Riah, W., Bureau, F., Gattin, I., Bodilis, J., 2014. Real-time PCR for quantification in soil of glycoside hydrolase family 6 cellulase genes. Lett. Appl. Microbiol. 59, 284–291.
10. Vacher, C., Hampe, A., Porté, A.J., Sauer, U., Compant, S., Morris, C.E., 2016. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. Annu. Rev. Ecol. Evol. Syst. 47, 1–24.
11. Compant, S., Samad, A., Faist, H., Sessitsch, A., 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J. Adv. Res. 19, 29–37.
12. Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and Functions of the Bacterial Microbiota of Plants. Annu. Rev. Plant Biol. 64, 807–838.
13. Hartmann, A., Fischer, D., Kinzel, L., Chowdhury, S.P., Hofmann, A., Baldani, J.I., Rothballer, M., 2019. Assessment of the structural and functional diversities of plant microbiota: Achievements and challenges – A review. J. Adv. Res. 19, 3–13.
14. Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9, 1473.
15. Chandran, H., Meena, M., Swapnil, P., 2021. Plant Growth-Promoting Rhizobacteria as a Green Alternative for Sustainable Agriculture. Sustainability 13(19), 10986.
16. Saggese, A., De Luca, Y., Baccigalupi, L., Ricca, E., 2022. An antimicrobial peptide specifically active against Listeria monocytogenes is secreted by *Bacillus pumilus* SF214. BMC Microbiol 22, 3.

17. Wang, N.R., Wiesmann, C.L., Melnyk, R.A., Hossain, S.S., Chi, M.-H., Martens, K., Craven, K., Haney, C.H., 2022. Commensal *Pseudomonas fluorescens* Strains Protect *Arabidopsis* from Closely Related *Pseudomonas* Pathogens in a Colonization-Dependent Manner. MBio 13, e02892-21.
18. Khanna, K., Jamwal, V.L., Sharma, A., Gandhi, S.G., Ohri, P., Bhardwaj, R., Al-Huqail, A.A., Siddiqui, M.H., Marraiki, N., Ahmad, P., 2019. Evaluation of the role of rhizobacteria in controlling root-knot nematode infection in *Lycopersicon esculentum* plants by modulation in the secondary metabolite profiles. AoB Plants 11(6), plz069.
19. Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M.S., Grotewold, E., 2015. Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. Plant Signal. Behav. 10, e1027850.
20. Gruau, C., Trotel-Aziz, P., Villaume, S., Rabenoelina, F., Clément, C., Baillieul, F., Aziz, A., 2015. *Pseudomonas fluorescens* PTA-CT2 Triggers Local and Systemic Immune Response Against *Botrytis cinerea* in Grapevine. Mol Plant-Microbe Interact 28, 1117–1129.
21. Mathys, J., De Cremer, K., Timmermans, P., Van Kerkhove, S., Lievens, B., Vanhaecke, M., Cammue, B.P.A., De Coninck, B., 2012. Genome-Wide Characterization of ISR Induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 Against *Botrytis cinerea* Infection. Front. Plant Sci. 3, 108.
22. Slama, H.B., Cherif-Silini, H., Chenari Bouket, A., Qader, M., Silini, A., Yahiaoui, B., Alenezi, F.N., Luptakova, L., Triki, M.A., Vallat, A., Oszako, T., Rateb, M.E., Belbahri, L., 2019. Screening for Fusarium Antagonistic Bacteria From Contrasting Niches Designated the Endophyte *Bacillus halotolerans* as Plant Warden Against *Fusarium*. Front. Microbiol. 9, 3236.
23. Alam, M.K., Rahman, M.M., Jamal, A.H.M., Islam, M.R., 2021. Screening of antagonistic potential bacteria from rhizosphere soil against phytopathogenic fungi related to selected vegetable crops. J. Adv. Biotechnol. Exp. Ther. 4, 35–43.
24. Hu, W., Gao, Q., Hamada, M.S., Dawood, D.H., Zheng, J., Chen, Y., Ma, Z., 2014. Potential of *Pseudomonas chlororaphis* subsp. *aurantiaca* Strain Pcho10 as a Biocontrol Agent Against *Fusarium graminearum*. Phytopathology 104, 1289–97.
25. Wright, E.S., Yilmaz, L.S., Noguera, D.R., 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Applied and Environmental Microbiology. 78(3), 717–725.
26. Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38(7), 3022–3027.
27. Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12), 1647–1649.
28. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41(D1), D590-D596.
29. Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal Database Project: data and tools for high throughput rRNA

analysis. Nucleic Acids Res. 42, D633-D642.

30. Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10(3), 512–526.
31. Ortiz-Castro, R., Campos-García, J., López-Bucio, J., 2013. Rapid identification of plant-growth-promoting rhizobacteria system with Arabidopsis. Molecular Microbial Ecology of the Rhizosphere. Frans J. De Bruijn (Ed). Vol I. Wiley-Blackwell, New Jersey, pp.345–353.
32. Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Rio, R.E., Campos-García, J., López-Bucio, J., 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc. Natl. Acad. Sci. USA 108(17), 7253–7258.
33. Tzec-Interián, J.A., Desgarennes, D., Carrión, G., Monribot-Villanueva, J.L., Guerrero-Analco, J.A., Ferrera-Rodríguez, O., Santos-Rodríguez, D.L., Liahut-Guin, N., Caballero-Reyes, G.E., Ortiz-Castro, R., 2020. Characterization of plant growth-promoting bacteria associated with avocado trees (*Persea americana* Miller) and their potential use in the biocontrol of *Scirtothrips perseae* (avocado thrips). PLoS One 15(4), e0231215.
34. Carreras-Villaseñor, N., Rodríguez-Haas, J.B., Martínez-Rodríguez, L.A., Pérez-Lira, A.J., Ibarra-Laclette, E., Villafán, E., Castillo-Díaz, A.P., Ibarra-Juárez, L.A., Carrillo-Hernández, E.D., Sánchez-Rangel, D., 2022. Characterization of two *Fusarium solani* species complex isolates from the ambrosia beetle *Xylosandrus morigerus*. J. Fungi 8, 231.
35. Báez-Vallejo, N., Camarena-Pozos, D.A., Monribot-Villanueva, J.L., Ramírez-Vázquez, M., Carrión-Villarnovo, G.L., Guerrero-Analco, J.A., Partida-Martínez, L.P. and Reverchon F., 2020. Forest tree associated bacteria for potential biological control of *Fusarium solani* and of *Fusarium kuroshium*, causal agent of Fusarium dieback. Microbiological Research, 235, 126440.
36. Cazorla, F.M., Mercado-Blanco, J., 2016. Biological control of tree and woody plant diseases: an impossible task? BioControl, 61, 233–242.
37. Kong, W.L., Li, P.S., Wu, X.Q., Wu, T.Y., Sun, X.R., 2020. Forest tree associated bacterial diffusible and volatile organic compounds against various phytopathogenic fungi. Microorganisms 8(4), 590.
38. Reverchon, F., Escudero-Osorio, Y.S., Morteo-Zavaleta, J., Guevara-Avendaño, E., Ramírez-Vázquez, M., 2020. Inhibición de *Fusarium solani* por bacterias de la filósfera y rizósfera de árboles del bosque mesófilo de montaña. Biotecnología y Sustentabilidad, 5(1), 3–18.
39. Mayer, E., de Quadros, P.D., Fulthorpe, R., Cann, I., 2019. *Plantibacter flavus*, *Curtobacterium herbarum*, *Paenibacillus taichungensis*, and *Rhizobium selenitireducens* Endophytes Provide Host-Specific Growth Promotion of *Arabidopsis thaliana*, Basil, Lettuce, and Bok Choy Plants. Appl. Environ. Microbiol. 85, e00383-19.
40. Pandey, N., 2020. Exiguobacterium. In: Amaresan, N., Senthil Kumar, M., Annapurna, K., Kumar, K., Sankaranarayanan, A., Editors. Benef. Microbes Agro-Ecology, Academic Press; pp. 169–183.
41. Santos, R.M.d., Rigobelo, E.C., 2021. Growth-Promoting Potential of Rhizobacteria Isolated From Sugarcane. Front. Sustain. Food Syst. 5, 596269.

42. Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F., Hashem, A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front. Microbiol. 8, 2104.
43. Ulfa, R., Suryanto, D., Munir, E., 2018. An ability of endophytes from blackboard tree (Alstonia scholaris) in increasing chili performance. J. Pure Appl. Microbiol. 12, 815–825.
44. Niu, B., Paulson, J.N., Zheng, X., Kolter, R., 2017. Simplified and representative bacterial community of maize roots. Proc. Natl. Acad. Sci. 114, E2450-E2459.
45. Kavuthodi, B., Sebastian, D., 2018. Review on bacterial production of alkaline pectinase with special emphasis on *Bacillus* species. Biosci. Biotechnol. Res. Commun. 11, 18–30.
46. Shankar, T., Sankaralingam, S., Balachandran, C., Chinnathambi, A., Nasif, O., Ali Alharbi, S., Park, S., Baskar, K., 2021. Purification and characterization of carboxymethylcellulase from *Bacillus pumilus* EWBCM1 isolated from earthworm gut (*Eudrilus eugeniae*). J. King Saud Univ. – Sci. 33, 101261.
47. Iskandar, C.F., Borges, F., Taminiau, B., Daube, G., Zagorec, M., Remenant, B., Leisner, J.J., Hansen, M.A., Sorensen, S.J., Mangavel, C., Cailliez-Grimal, C., Revol-Junelles, A.M. 2017. Comparative Genomic Analysis Reveals Ecological Differentiation in the Genus *Carnobacterium*. Front. Microbiol. 8, 357.
48. Berlemont, R., Martiny, A.C., Glycoside Hydrolases across Environmental Microbial Communities. PLOS Comput. Biol. 12, e1005300.
49. Ruiu, L., 2015. Insect Pathogenic Bacteria in Integrated Pest Management. Insects 6, 352–367.
50. Cho, H., Park, J.Y., Kim, Y.K., Sohn, S.-H., Park, D.S., Kwon, Y.-S., Kim C.-W., Back C.-G., 2019. Whole-genome sequence of *Erwinia persicina* b64, which causes pink soft rot in onions. Microbiol. Resour. Announc. 8(1), e01302-18.
51. Busby, J.N., Landsberg, M.J., Simpson, R.M., Jones, S.A., Hankamer, B., Hurst, M.R.H., Lott, J.S., 2012. Structural analysis of Chi1 Chitinase from Yen-Tc: The multisubunit insecticidal ABC toxin complex of *Yersinia entomophaga*. J. Mol. Biol. 415, 359–371.
52. Hugouvieux-Cotte-Pattat, N., Condemine, G., Shevchik, V.E., 2014. Bacterial pectate lyases, structural and functional diversity. Environ. Microbiol. Rep. 6, 427–440.
53. Samanta, S., 2019. Microbial pectinases: a review on molecular and biotechnological perspectives. J. Microbiol. Biotechnol. FOOD Sci. 9, 248–66.
54. Paulson, A.R., O'Callaghan, M., Zhang, X.-X., Rainey, P.B., Hurst, M.R.H., 2021. *In vivo* transcriptome analysis provides insights into host-dependent expression of virulence factors by *Yersinia entomophaga* MH96, during infection of *Galleria mellonella*. G3 Genes Genomes Genetics 11, jkaa024.
55. Nascimento, F., Vicente, C., Cock, P., Tavares, M., Rossi, M., Hasegawa, K., Mota, M., 2018. From plants to nematodes: *Serratia grimesii* BXF1 genome reveals an adaptation to the modulation of multi-species interactions. Microb. Genomics 4(7), 178.

Tables

Table 1 is available in the Supplementary Files section.

Figures

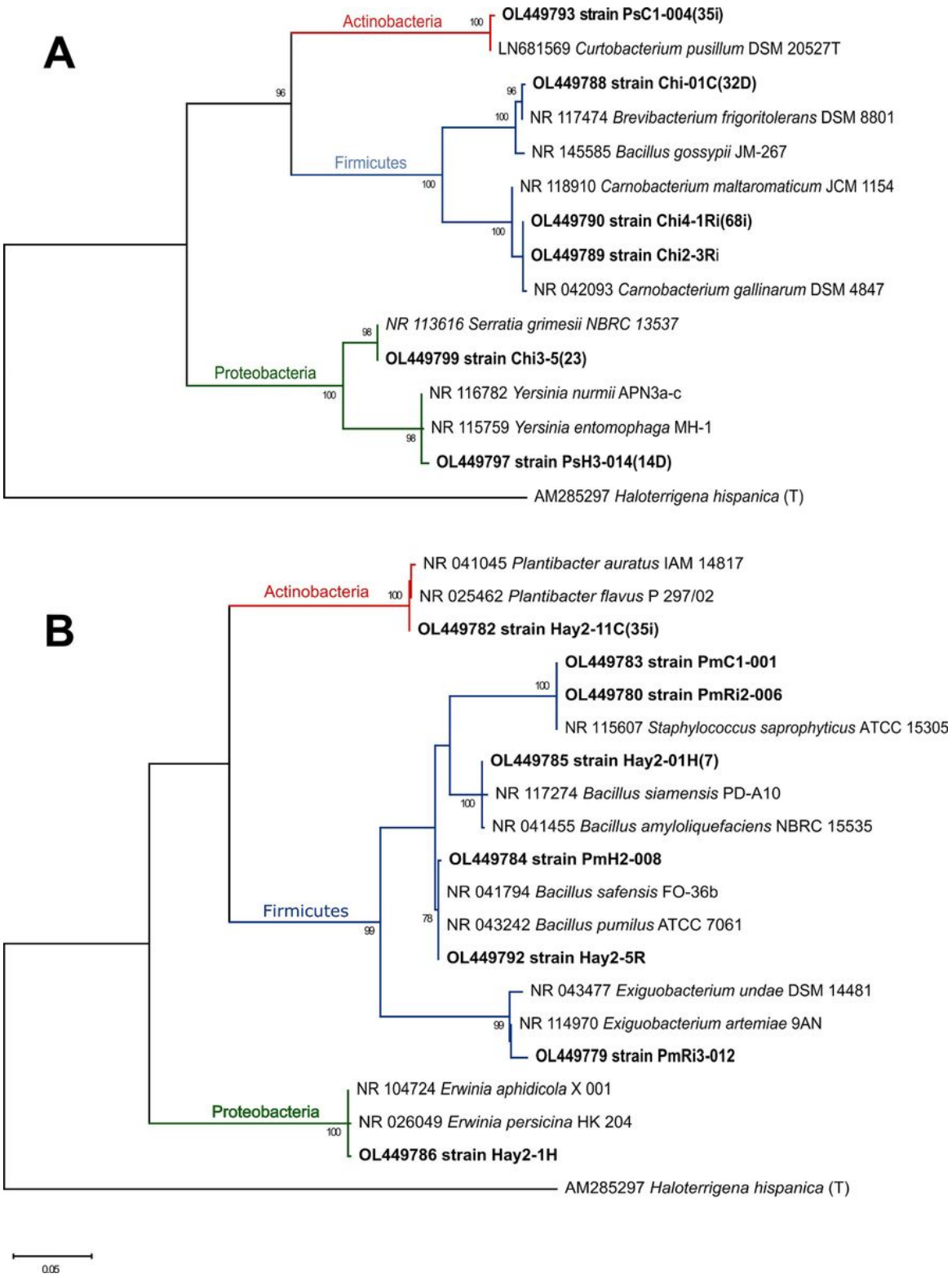

Figure 1

Phylogenetic tree of the 16S rRNA sequence of bacteria isolated from *Persea schiedeana*and *Platanus mexicana* trees. A) *Persea schiedeana, B) Platanus mexicana. Haloterrigena hispanica was used as outer group.*

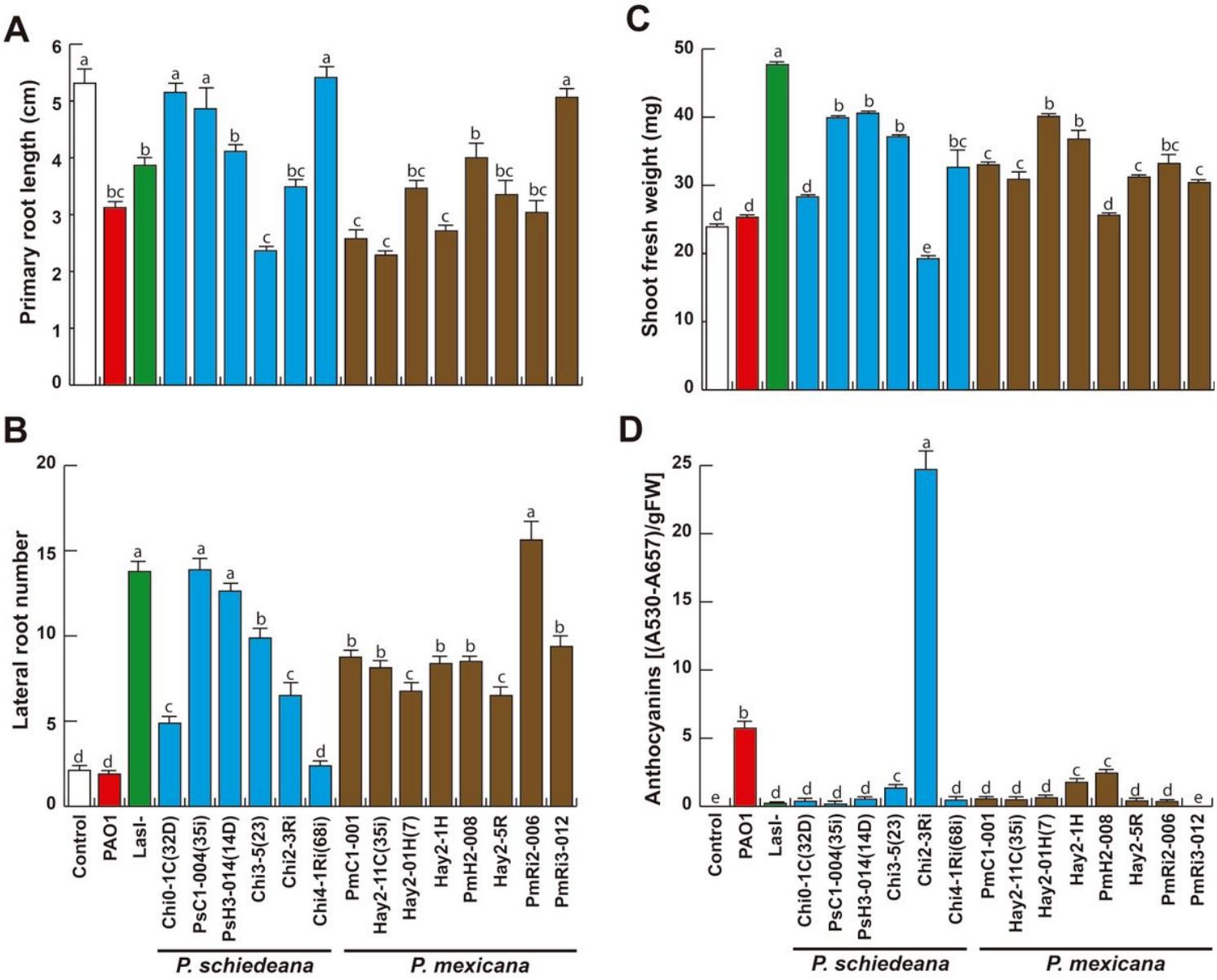

Figure 2

Effects of bacterial strains isolated from *P. schiedeana* and *P. mexicana* to the *in vitro* Arabidopsis-bacteria co-culture system. Arabidopsis seedlings of 5 days after germination were co-culture with bacterial strains and growth further 8 d. A) Primary root length, B) Lateral root numbers, C) Shoot fresh weight, D) Anthocyanins. Data represent mean ± standard deviation (n=24). Different letters represent the statistical difference at $p<0.05$.

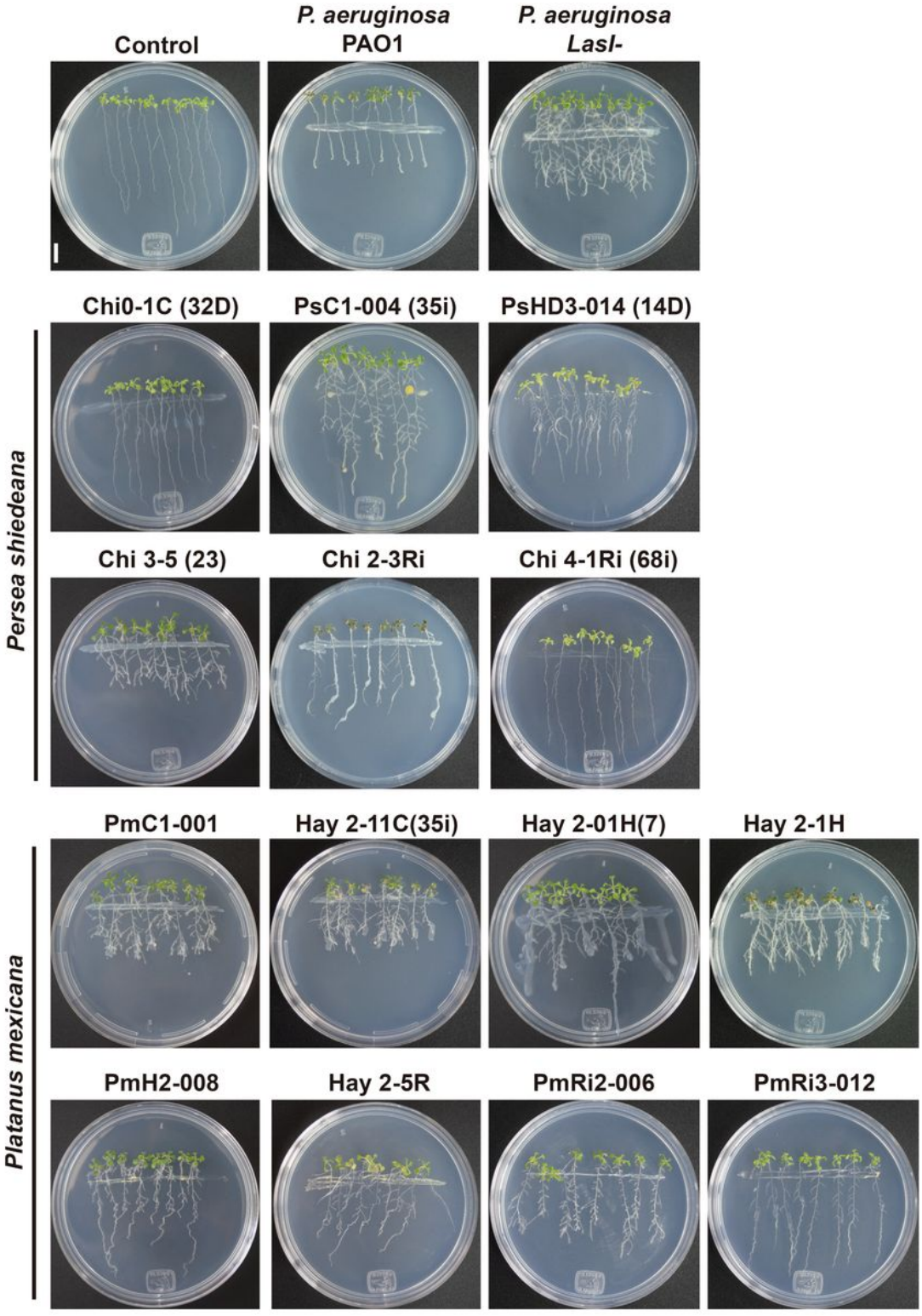

Figure 3

Arabidopsis growth responses to bacterial strains into *in vitro* co-culture system with bacterial strains. Arabidopsis seedlings were growth on 0.2x MS agar-medium and then were co-cultivated with the bacterial strains isolated from *Persea schiedeana* and *Platanus mexicana* or un-inoculated (control treatment). *Pseudomonas aeruginosa* PAO1 (Wild-Type) and *P. aeruginosa LasI-* (a quorum-sensing mutant affected in the LasI synthase) were used as pathogenic and plant growth-promoting strains

respectively. Representative pictures of Arabidopsis growth responses to bacterial strains. Scale bar = 1cm.

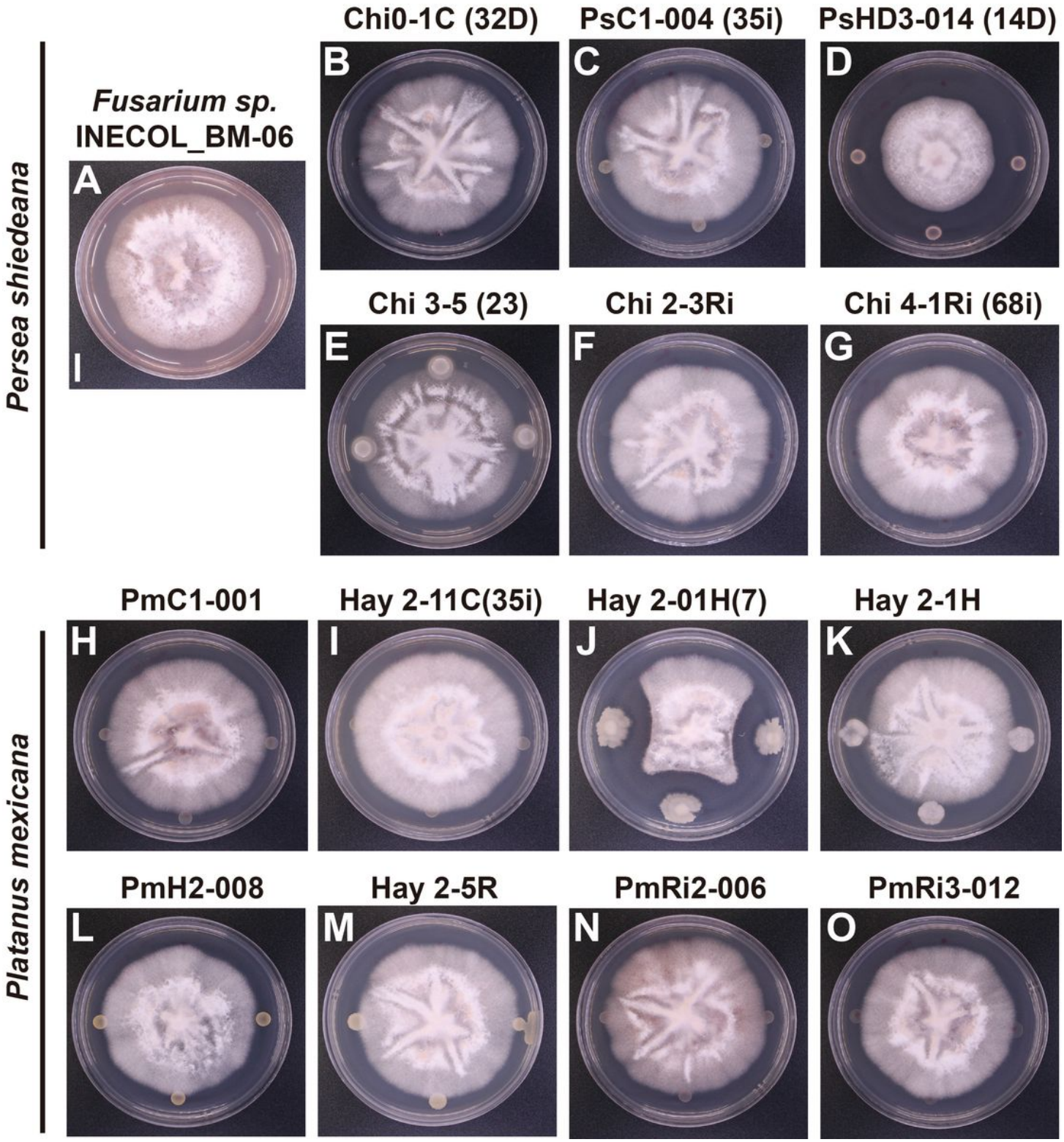

Figure 4

Co-culture of bacterial strains and *Fusarium sp.* strain INECOL_BM-06. A) *Fusarium sp.* strain INECOL_BM-06. Strains isolated from *Persea shiedeana*: B) Chi 0-1C (32D), C) PsC1-004 (35i), D) PsH3-

014(14D), E) Chi 3-5(23), F) Chi 2-3Ri, G) Chi 4-1Ri (68i). Strains isolated from *Platanus mexicana*: H) PmC1-001, I) Hay 2-11C (35i), J) Hay 2-01H (7), K) Hay 2-1H, L) PmH2-008, M) Hay 2-5R, N) PmRi2-006, O) PmRi3-012. Scale bar = 1cm.

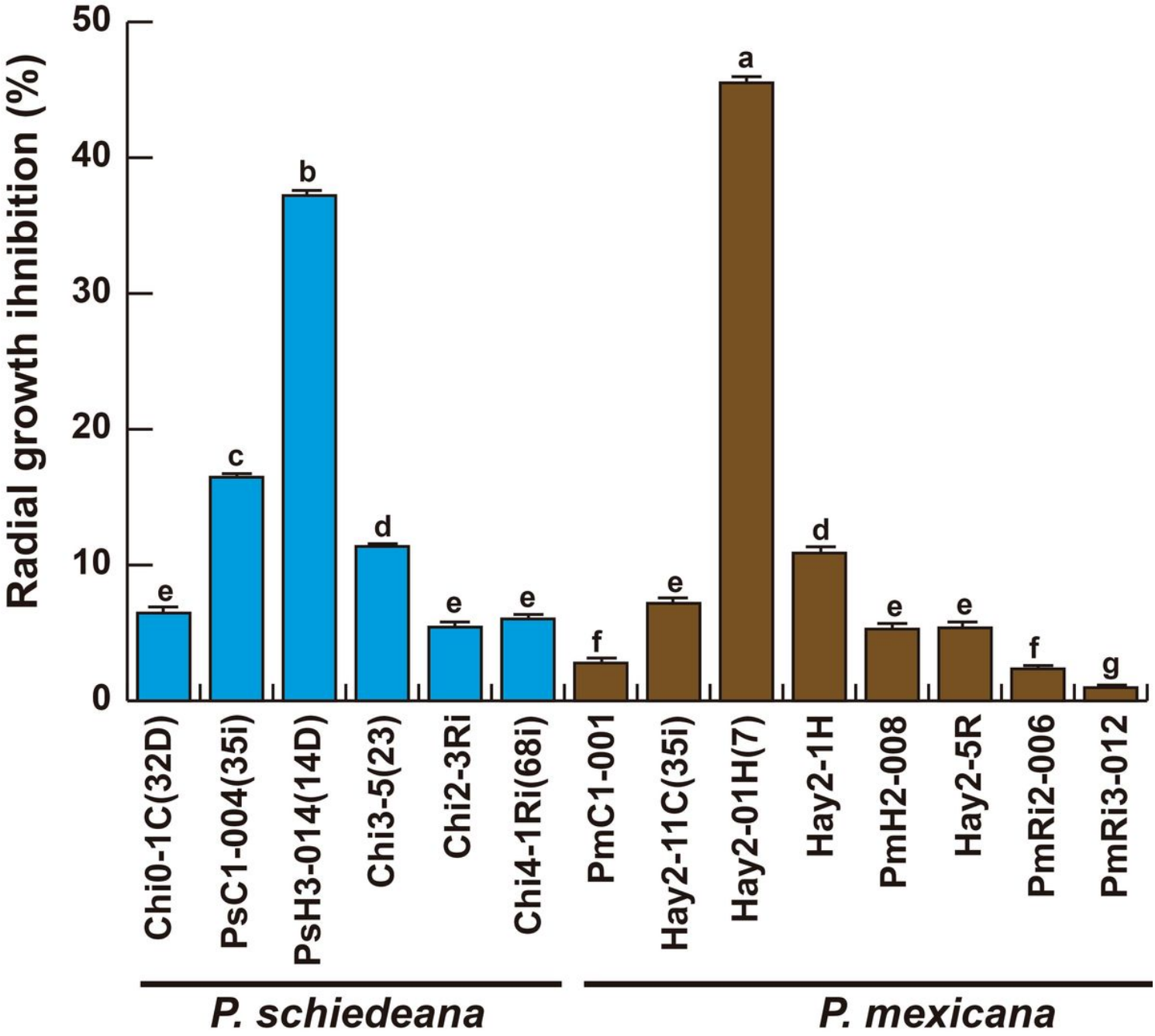

Figure 5

Effects of bacterial strains isolated from *P. shiedeana* and *P. mexicana* on the growth inhibition of *Fusarium* sp. strain INECOL_BM-06. Percentage of *Fusarium sp.* strain INECOL_BM-06. growth inhibition caused by the bacterial strain antagonistic activities. Data represent mean ± standard deviation (n=3). The experiment was repeated twice times with similar results.

Supplementary Files

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- Table1.jpg